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Association of FTO genotype with obesity and bone health among community-dwelling adults ; Goto Island study on bone health

Xu Xiao, MD[a], Satoshi Mizukami, PhD[a], Kazuhiko Arima[a], MD, PhD[a], Kyoya Morikawa[a], Midori Motoi, PhD[b], Naoki Iwamoto, MD, PhD[c], Yoshihito Tomita, PhD[d], Michiko Uchiyama, MD[a], Yuzo Honda, MD[a], Natsumi Tanaka, MD, PhD[e], Hisashi Goto MD, PhD[f], Maiko Hasegawa MD, PhD[g], Youko Sou MD, PhD[h], Ritsu Tsujimoto, MD, PhD[i], Takayuki Nishimura, PhD[j], Shin-ya Kawashiri, MD, PhD[c, k], Mami Tamai, MD, PhD[c], Hirotomo Yamanashi, MD, PhD[l, m], Yasuyo Abe, MD, PhD[n], Tomoki Origuchi, MD, PhD[o], Yasuhiro Nagata, MD, PhD[k], Atsushi Kawakami, MD, PhD[c], Takahiro Maeda, MD, PhD[l], Kiyoshi Aoyagi, MD, PhD[a]

[a] Department of Public Health, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

[b] Department of Living Business, Seika Women's Junior College, Fukuoka, Japan

[c] Department of Immunology and Rheumatology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

[d] School of Rehabilitation, Department of Physical Therapy, Tokyo Professional University of Health Sciences, Tokyo, Japan

[e] Department of Orthopedic Surgery, Nagasaki Rosai Hospital, Nagasaki, Japan

[f] Ken-Hoku Health Care Office, Nagasaki, Japan

[g] Medical Policy Division, Nagasaki Prefectural Government, Nagasaki, Japan

[h] National Health Insurance & Health Improvement Division, Nagasaki Prefectural Government, Nagasaki, Japan

[i] Department of Orthopedic Surgery, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

[j] Department of Human Science, Faculty of Design, Kyushu University, Fukuoka, Japan

[k] Department of Community Medicine, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

[l] Department of General Medicine, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

[m] Department of Clinical Medicine, Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan

[n] Department of Health and Nutrition, Nishikyusyu University, Saga, Japan.

[o] Department of Rehabilitation Sciences, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

Bone mass is tuned by various factors, including aging, menopause, low body weight, and genetic variations. Here, we showed an independent association between a genotype on the fat mass- and obesity-associated FTO gene (#610966 on OMIM) and bone loss after adjusting for age and body mass index (BMI). A cross-sectional study was nested in a prospective observational study of 1,828 participants (median age: 69 [62-76] years in men and 68 [61-75] years in women) residing in a rural city in western Japan (Goto Island study). Participants were recruited during medical checkups in 2014 and 2016 from the community-dwelling population. The bone mass of the calcaneus was evaluated using quantitative ultrasound. The single nucleotide polymorphism (SNP) rs1421085 was genotyped using a hydrolysis probe. The chi-squared test was used to determine whether the variants were in equilibrium in this population. There were differences in medians of BMI among the genotypes (24.3 in CC, 23.0 in CT, and 22.6 in TT, P = 0.01), but not in those of bone mass. There was a significant association between the minor allele (C) and being overweight in a gene dosage-dependent manner (BMI > 25, OR per allele =1.52, 95% CI = 1.07-2.14, P = 0.02 in men, OR = 1.48, 95% CI = 1.16-1.95, P = 0.01 in women). Logistic regression analysis showed a significant protective association in male carriers of the minor allele against low bone mass (QUS T-score less than -2.0) after adjusting for age and BMI in men aged 65-75 years (OR = 0.50, 95% CI = 0.27-0.96, P = 0.036), with no significant association in women.

Address correspondence: Kazuhiko Arima, MD, PhD
Department of Public Health, Nagasaki University Graduate School of Biomedical Sciences, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan
E-mail: kzarima-ngs@umin.ac.jp
Tel: +81 95 819 7067, Fax: +81 95 819 7069

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Our study indicated an association between the genetic polymorphism of FTO and bone mass among community-dwelling men aged 65-75 years. The polymorphism may play a role in bone health with higher BMI and other beneficial functions.

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Key words: Bone ultrasound, Osteoporosis, General population studies, Human association studies, FTO gene

Introduction

One of the components of bone is mineralized bone tissue with a rigid honeycomb internal structure. Osteoporosis occurs due to an imbalance between bone resorption and bone formation[1]. Osteoporosis has become a serious public health problem in the aging population. The occurrence of osteoporosis is related to genetic factors and the external environment and is an important cause of mortality and morbidity in the elderly. In a report of a simulation projection model over a 20-year period from 2020 to 2040 in Japan, estimated total number of fractures summed to 21.6 million at a total cost of US $410.2 billion[2]. The clinical consequences and economic burden of the disease call for measures to assess high risk groups for appropriate interventions[3].

Quantitative ultrasound (QUS) technology has emerged as a convenient tool for screening of fracture risk. Although QUS cannot be used to diagnose osteoporosis or osteopenia in terms of the WHO definition and of the Japanese Society of Bone Metabolism and the Japanese Society of Osteoporosis jointly published the diagnostic criteria for primary osteoporosis (2012 revision)[4], several studies have also indicated that it can predict fractures in men[5], in women[6, 7], and in both genders[8, 9]. A meta-analysis including 21 studies, which included 55,164 women and 13,742 men, revealed significant association between QUS parameters and risk of various fracture outcomes[10]. Among QUS parameters, stiffness index (SI) was considered to be reliable indicators of bone health [11, 12, 13].

Bone mass is tuned by various factors, including aging, menopause, low body weight, and genetic variations. Most of the basic characteristics (age, gender, BMI) are variable and have interrelated effects; therefore, it might be difficult to analyze the independent relevance of bone health with the variable basic characteristics. Genotype is the collection of genes responsible for the various genetic traits of a given organism. Genotype refers specifically to the genes, not the traits; that is, the raw information in an organism's DNA, which is an unchangeable characteristic against diseases. The genetic factors associated with osteoporosis are those genes that determine bone quality, size, structure, microstructure, and intrinsic bone properties. There have been a few candidate genes that that have been reported to regulate bone mass[14].

FTO is located in the 16q12.2 chromosomal region and contains nine exons. The FTO protein has a molecular mass of 58 kDa, is widely expressed in fetal and adult tissues, and is mostly distributed in the brain, especially in the hypothalamus; however, its specific function and mechanism have not been fully elucidated. In 2007, Frayling et al. showed a relationship between FTO and BMI[15]. This gene has been reported to be involved in various conditions, including colorectal cancer[16], pancreatic cancer[17, 18], breast cancer[19], and depression[20].

A recent study found that mice with systemic FTO knockout showed significant postnatal growth retardation compared to the control group. They were shorter in length, lighter in weight, and had a lower bone density[21]. Another study by Claussnitzer using endogenous CRISPR-Cas9 genome editing showed a critical role for the rs1421085 variant in the adipocyte thermogenesis regulation pathway through interaction with the ARID5B, IRX3, and IRX5 genes, thus affecting adipocyte differentiation. Their results indicated that the rs1421085 T- to-C single-nucleotide alteration underlies the association between FTO and obesity[22].

From the results of these studies, FTO may be a multipotent genetic factor that not only affects the obesity phenotype, but also affects the osteoporotic phenotype in conditional knockout mice[21]. Given the evidence of pleiotropic effects, we hypothesized that variants in FTO are associated with osteoporosis risk. We assessed the associations of the FTO genotype with obesity and bone health among community-dwelling adults, which would lead to improved quality of bone health evaluation.

Materials and Methods

Study design

A cross-sectional study of 1,828 participants (median age: 69 [62-76] years in men and 68 [61-75] years in women) was nested in a prospective cohort in a rural city in western Japan[23]. The participants volunteered to partake in this cross-sectional study. Written consent forms were available in Japanese to ensure a comprehensive understanding, and informed consent was obtained from all participants. This study was approved by the Ethics Committee for Human Use of Nagasaki University

(project registration number 14051404). The survey was targeted at population residing in rural communities in Goto city in western Japan who underwent a general medical check-up in 2014 and 2016, as recommended by the Japanese government. Participants who had inadequate cognitive functioning to answer the questionnaire were included neither in this study nor in this analysis.

Measurements

The BMI (kg/m^2) was calculated by measuring the body weight and height of patients wearing lightweight clothing using an automatic body composition analyzer (BF-220; Tanita, Tokyo, Japan).

Broadband ultrasound attenuation (BUA; dB/MHz) and the speed of sound (SOS; m/s) through the right calcaneal bone were measured using QUS (Achilles InSight, GE Lunar Corp., Madison, WI, USA). The calcaneal stiffness index (SI), a function of BUA and SOS, was automatically calculated by using the scanner software according to the following formula: SI = (0.67 × BUA) + (0.28 × SOS) - 420. The validations for the same model of QUS were reported (a coefficient of variation (CV) of 0.4% for SOS, 3.0% for BUA stiffness obtained with Achilles InSight)[24]. We obtained similar values as the validations (a CV of 0.4% for SOS, 1.9% for BUA, and 3.3% for stiffness as intra-assay coefficients, a CV of 0.3% for SOS, 0.7% for BUA, and 1.7% for stiffness as inter-assay coefficients). Groups of low bone mass were defined as a subject with a figure of < -1.0, < -2.0, < -3.0, because of previous longitudinal studies which reported fracture risks for one decrement in QUS T-score[6-10, 12]. Additionally, one was defined as a subject with a figure of < -2.5 in QUS T-score of SI, because of the definition for osteoporosis by WHO, which includes a figure of < -2.5 in BMD T-score.

Genomic DNA was obtained from peripheral blood samples using Gene Prep Star NA-480 (Kurabo Industries Ltd., Osaka, Japan). The SNP rs1421085 was genotyped using the TaqMan SNP Genotyping Assay kit (C___8917103, Thermo Fisher Scientific, Tokyo, Japan) on a LightCycler 480 instrument (Roche Diagnostics, Basel, Switzerland). Briefly, genomic DNA was amplified by PCR (95 ℃ for 30 s, 40 cycles between 95 ℃ for 5 s and 60 ℃ for 30 s, and 50 ℃ for 30 s) combined with both types of quenched fluorogenic hydrolysis probes (VIC/FAM). No increase in fluorescence intensity was observed in each PCR cycle compared with the negative control containing no template DNA.

Statistical Analysis

A total of 1,828 participants (650 men and 1,178 women) aged 27–97 years were included in our analysis. The normality of variables was assessed using the Kolmogorov-Smirnov test. The Mann-Whitney U test was used to compare differences in the medians of numerical variables, and the chi-square (χ^2) test was used to compare the distribution of categorical variables between the two groups. The Kruskal-Wallis test was used to compare medians among the three genotype groups. Multiple linear regression analysis was used to explore the estimated effect size by the risk allele on the calcaneal SI, adjusting for age and BMI. Logistic regression analysis was applied to explore the independent association of FTO genotype on being overweight and having low bone mass with adjustment for age and BMI.

Statistical significance was set at $P < 0.05$. All statistical analyses were performed using the SPSS software version 23 (SPSS Inc., Chicago, IL, USA).

Result

Table 1 shows the characteristics of the 1,828 participants. The median (interquartile range [IQR]) ages of the men and women were 69 (62-76) and 68 (61-75) years, respectively. The medians of weight, height, BMI, and QUS parameters were greater in men than in women ($P < 0.001$ for all).

The characteristics of each FTO genotype group are shown in Table 2. The Kruskal-Wallis test revealed significant differences in the medians of weight (59.8 [49.2-68.7] of the CC group, 55.5 [48.8-63.9] in the CT group, and 53.9 [48.3-61.9] of the TT group, $P = 0.004$) and BMI (24.4 [21.3-27.0] in the CC group, 22.8 [20.6-25.2] of the CT group, 22.4 [20.3-24.6] of the TT group, $P < 0.001$), but there was no significant difference in QUS parameters among the three groups. Additionally, a linear regression analysis showed an estimated effect size of one risk allele in this genotype towards the values of BMI (unstandardized B: 0.69, 95% CI: 0.25-1.14, $P = 0.002$ in men and B: 0.45, 95% CI: 0.07-0.82, $P = 0.019$ in women), although the variables were not normally distributed. There were significant associations between the minor allele and being overweight (BMI of > 25, OR=1.52, 95% CI = 1.07-2.14, $P = 0.02$ in men; OR = 1.48, 95% CI = 1.16-1.95, $P = 0.01$ in women).

Out of the 1,828 participants, 1,374 (75.2%) had bone mass of QUS T-score less than -1.0, 968 (53.0%) participants had a bone mass of QUS T-score less than -2.0 and 449 (24.6%) participants had a bone mass of QUS T-score less

than -3.0. Logistic regression was applied to evaluate the association between genotype and groups with low bone mass. There was a significant independent association between the genotype and group with QUS T-score less than -2.0 among participants of all ages in men after an adjustment for age and BMI (OR: 0.67, 95% CI: 0.47-0.97, P = 0.031), but not in women (Table 3).

After stratifying by age and gender, using univariate analysis, χ^2 tests showed significant differences in the distribution of the genotype in men aged 40-64 years against QUS T-score less than -1.0 (27/60 vs. 86/138, $\chi^2 = 5.12$, P = 0.029) and in men aged 65-75 years against QUS T-score less than -2.0 (16/73 vs. 65/174, $\chi^2 = 5.56$, P = 0.018), but not in women (Table 4).

Using multivariate analysis with adjustments, logistic regression analysis showed a significant protective association with carriers of the risk allele against the group with QUS T-score less than -2.0 after adjusting for age and BMI in men aged 65-75 years (OR = 0.50, 95% CI = 0.27-0.96, P = 0.036) and one against the group with QUS T-score less than -1.0 in men aged 40-64 years (OR = 0.52, 95% CI = 0.28-0.98, P = 0.043 in supplemental table 1), but no significant association was found in women (Table 5). Additionally, there was no significant association against the group with QUS T-score less than -2.5 or -3.0 in both genders (supplemental table 2, 3).

Table 1. Characteristics of the study participants by gender.

Variable	Men (n=650)	Women (n=1, 178)	p-value
Age (year)	69 [62 -76]	68 [61 -75]	0.29
Weight (kg)	63.5 [57.0 - 69.6]	51.0 [45.5 - 56.2]	< 0.001
Height (cm)	164.0 [160.0 - 168.6]	152.4 [148.3 - 156.2]	< 0.001
BMI (kg/㎡)	23.6 [21.6 - 25.5]	21.9 [19.9 - 24.3]	< 0.001
QUS SI	85.4 [75.2 - 97.3]	67.8 [59.6 - 77.9]	< 0.001
QUS T-score	-1.6 [-2,4 - -0.6]	-2.4 [-3.2 - -1.4]	< 0.001
QUS T<-1.0, n (%)	412 (63.4)	962 (81.7)	< 0.001
QUS T<-2.0, n (%)	247 (38.0)	721 (61.2)	< 0.001
QUS T<-3.0, n (%)	83 (12.8)	388 (31.1)	< 0.001

Data are shown as medians [interquartile range] or n (%). BMI: body mass index, QUS: quantitative ultrasound, SI: stiffness index, Mann-Whitney U test or χ^2 test for medians between genders.

Table 2. Characteristics of the study participants by genotype.

	CC (n=44)	CT (n=507)	TT (n=1, 277)	p-value
Age (year)	66 [60-75]	68 [62-76]	68 [62-76]	0.77
Weight (kg)	59.8 [49.2-68.7]	55.5 [48.8-63.9]	53.9 [48.3-61.9]	0.004
Height (cm)	155.0 [150.7-162.6]	156.0 [151.1-162.0]	155.7 [150.4-161.9]	0.88
BMI (kg/㎡)	24.4 [21.3-27.0]	22.8 [20.6-25.2]	22.4 [20.3-24.6]	< 0.001
QUS SI	75.4 [62.7 - 88.4]	73.7 [63.3 - 88.4]	73.4 [63.0 - 86.3]	0.55
QUS T-score	-2.0 [-3.0 - -1.0]	-2.0 [-2,9 - -0.9]	-2.1 [-3.0 - -1.1]	0.62
QUS T<-1.0, n (%)	33 (75.0)	369 (72.8)	972 (76.1)	0.34
QUS T<-2.0, n (%)	22 (50.0)	258 (50.9)	688 (53.9)	0.48
QUS T<-3.0, n (%)	11 (25.0)	120 (23.7)	318 (24.9)	0.86

Data are shown as medians [interquartile range] or n (%). BMI: body mass index, QUS: quantitative ultrasound, SI: stiffness index, Kruskal-Wallis test for medians among genotype groups.

Table 3. Odds ratio of risk factors for low bone mass on the QUS T-score.

Gender	QUS T-score	Variables	Unit	OR	95%CI	p-value
Men	QUS T<-1.0	Age (year)	1	1.04	(1.02-1.05)	< 0.001
		BMI (kg/㎡)	1	0.95	(0.90-1.00)	0.051
		FTO (CC&CT)	TT (reference)	0.81	(0.57-1.15)	0.23
	QUS T<-2.0	Age (year)	1	1.05	(1.03-1.06)	< 0.001
		BMI (kg/㎡)	1	0.94	(0.89-1.00)	0.039
		FTO (CC&CT)	TT (reference)	0.67	(0.47-0.97)	0.031*
	QUS T<-3.0	Age (year)	1	1.09	(1.06-1.12)	< 0.001
		BMI (kg/㎡)	1	0.90	(0.83-0.98)	0.019
		FTO (CC&CT)	TT (reference)	0.78	(0.45-1.35)	0.38
Women	QUS T<-1.0	Age (year)	1	1.12	(1.10-1.14)	< 0.001
		BMI (kg/㎡)	1	0.94	(0.90-0.98)	0.009
		FTO (CC&CT)	TT (reference)	0.93	(0.64-1.34)	0.68
	QUS T<-2.0	Age (year)	1	1.10	(1.09-1.12)	< 0.001
		BMI (kg/㎡)	1	0.90	(0.87-0.94)	< 0.001
		FTO (CC&CT)	TT (reference)	1.11	(0.83-1.49)	0.47
	QUS T<-3.0	Age (year)	1	1.11	(1.09-1.13)	< 0.001
		BMI (kg/㎡)	1	0.93	(0.89-0.97)	0.001
		FTO (CC&CT)	TT (reference)	1.04	(0.77-1.40)	0.80

QUS: quantitative ultrasound, BMI: body mass index, OR: odds ratio, CI: confidence interval. *p < 0.05

Table 4. Univariate analysis of genotype for bone health in age groups in both genders.

Gender	Age	QUS T-score	FTO genotype		p-value
			CC&CT	TT	
Men	40-64		n=60	n=138	
		QUS T<-1.0, n (%)	27 (45.0)	86 (62.3)	χ^2=5.12, p=0.029*
		QUS T<-2.0, n (%)	13 (21.7)	48 (34.8)	χ^2=3.38, p=0.093
		QUS T<-3.0, n (%)	1 (1.7)	12 (8.7)	χ^2=3.37, p=0.11
	65-75		n=73	n=174	
		QUS T<-1.0, n (%)	44 (60.3)	108 (62.1)	χ^2=0.07, p=0.89
		QUS T<-2.0, n (%)	16 (21.9)	65 (37.4)	χ^2=5.56, p=0.018*
		QUS T<-3.0, n (%)	5 (6.8)	16 (9.2)	χ^2=0.36, p=0.63
	75<		n=63	n=126	
		QUS T<-1.0, n (%)	49 (77.8)	93 (73.8)	χ^2=0.35, p=0.60
		QUS T<-2.0, n (%)	35 (55.6)	68 (54.0)	χ^2=0.04, p=0.88
		QUS T<-3.0, n (%)	16 (25.4)	32 (25.4)	χ^2=0.00, p=1.00
Women	40-64		n=114	n=269	
		QUS T<-1.0, n (%)	70 (61.4)	176 (65.4)	χ^2=0.56, p=0.49
		QUS T<-2.0, n (%)	48 (42.1)	104 (38.7)	χ^2=0.40, p=0.57
		QUS T<-3.0, n (%)	13 (11.4)	32 (11.9)	χ^2=0.02, p=1.00
	65-75		n=129	n=306	
		QUS T<-1.0, n (%)	116 (89.9)	277 (90.5)	χ^2=0.04, p=0.86
		QUS T<-2.0, n (%)	87 (67.4)	203 (66.3)	χ^2=0.05, p=0.91
		QUS T<-3.0, n (%)	45 (34.9)	90 (29.4)	χ^2=1.27, p=0.26
	75<		n=95	n=235	
		QUS T<-1.0, n (%)	90 (94.7)	223 (94.9)	χ^2=0.01, p=1.00
		QUS T<-2.0, n (%)	80 (84.2)	196 (83.4)	χ^2=0.03, p=1.00
		QUS T<-3.0, n (%)	51 (53.7)	135 (57.4)	χ^2=0.39, p=0.54

QUS: quantitative ultrasound. *p < 0.05

Table 5. Multivariate analysis of genotype for low bone mass in participants separated into age groups for both genders.

Gender	Age	Variable	Unit	Univariate	multivariate
				OR (95%CI)	OR (95%CI)
Men	40-64	Age	1 increase		1.03 (0.99-1.08)
		BMI	1 increase		0.89 (0.80-0.99)
		FTO(CC&CT)	/TT	0.52 (0.26-1.05)	0.57 (0.28-1.18)
	65-75	Age	1		1.12 (1.02-1.23)
		BMI	1		1.01 (0.92-1.11)
		FTO(CC&CT)	/TT	0.47 (0.25-0.89)*	0.50 (0.27-0.96)*
	75<	Age	1		1.15 (1.06-1.25)
		BMI	1		0.96 (0.87-1.06)
		FTO(CC&CT)	/TT	1.07 (0.58-1.96)	1.08 (0.57-2.03)
Women	40-64	Age	1		1.15 (1.10-1.19)
		BMI	1		0.87 (0.81-0.94)
		FTO(CC&CT)	/TT	1.15 (0.74-1.80)	1.13 (0.69-1.83)
	65-75	Age	1		1.03 (0.96-1.11)
		BMI	1		0.89 (0.84-0.95)
		FTO(CC&CT)	/TT	1.05 (0.68-1.63)	1.15 (0.74-1.81)
	75<	Age	1		1.07 (0.98-1.16)
		BMI	1		0.96 (0.87-1.05)
		FTO(CC&CT)	/TT	1.06 (0.55-2.03)	1.02 (0.53-1.97)

Low bone mass: QUS T<-2.0, BMI: body mass index, OR: odds ratio, CI: confidence interval. Logistic regression analysis *P < 0.05

Table S1. Multivariate analysis of genotype for QUS T<-1.0 in participants separated into age groups for both genders.

Gender	Age	Variable	Unit	Univariate	multivariate
				OR (95%CI)	OR (95%CI)
Men	40-64	Age	1 increase		1.03 (0.99-1.08)
		BMI	1 increase		0.92 (0.84-1.01)
		FTO(CC&CT)	/TT	0.50 (0.27-0.91)*	0.52 (0.28-0.98)*
	65-75	Age	1		1.10 (1.01-1.21)
		BMI	1		0.98 (0.89-1.07)
		FTO(CC&CT)	/TT	0.93 (0.53-1.62)	1.00 (0.57-1.77)
	75<	Age	1		1.17 (1.05-1.31)
		BMI	1		0.98 (0.87-1.10)
		FTO(CC&CT)	/TT	1.24 (0.61-2.54)	1.25 (0.60-2.60)
Women	40-64	Age	1		1.18 (1.14-1.23)
		BMI	1		0.93 (0.87-1.00)
		FTO(CC&CT)	/TT	0.84 (0.53-1.32)	0.74 (0.44-1.24)
	65-75	Age	1		1.02 (0.91-1.14)
		BMI	1		0.93 (0.86-1.02)
		FTO(CC&CT)	/TT	0.93 (0.47-1.86)	0.99 (0.49-1.98)
	75<	Age	1		0.91 (0.81-1.03)
		BMI	1		0.88 (0.75-1.02)
		FTO(CC&CT)	/TT	0.97 (0.33-2.83)	1.10 (0.37-3.26)

BMI: body mass index, OR: odds ratio, CI: confidence interval. Logistic regression analysis *P < 0.05

Table S2. Multivariate analysis of genotype for QUS T<-3.0 in participants separated into age groups for both genders.

Gender	Age	Variable	Unit	Univariate	multivariate
				OR (95%CI)	OR (95%CI)
Men	40-64	Age	1 increase		1.07 (0.97-1.18)
		BMI	1 increase		0.93 (0.77-1.12)
		FTO(CC&CT)	/TT	0.18 (0.02-1.40)	0.19 (0.02-1.52)
	65-75	Age	1		1.19 (1.01-1.41)
		BMI	1		1.01 (0.86-1.18)
		FTO(CC&CT)	/TT	0.73 (0.26-2.06)	0.82 (0.28-2.36)
	75<	Age	1		1.19 (1.09-1.31)
		BMI	1		0.87 (0.77-0.98)
		FTO(CC&CT)	/TT	1.00 (0.50-2.00)	1.08 (0.51-2.30)
Women	40-64	Age	1		1.16 (1.08-1.25)
		BMI	1		0.85 (0.76-0.96)
		FTO(CC&CT)	/TT	0.95 (0.48-1.89)	0.90 (0.44-1.84)
	65-75	Age	1		1.02 (0.95-1.10)
		BMI	1		0.94 (0.88-1.00)
		FTO(CC&CT)	/TT	1.29 (0.83-1.99)	1.35 (0.87-2.10)
	75<	Age	1		1.09 (1.02-1.16)
		BMI	1		0.93 (0.87-1.00)
		FTO(CC&CT)	/TT	0.86 (0.53-1.39)	0.82 (0.50-1.34)

BMI: body mass index, OR: odds ratio, CI: confidence interval. Logistic regression analysis *P < 0.05

Table S3. Univariate analysis of genotype for QUS T<-2.5 in age groups in both genders.

Gender	Age	QUS T-score	FTO genotype		p-value
			CC&CT	TT	
Men	40-64		n=60	n=138	
		QUS T<-2.5, n (%)	6 (10.0)	26 (18.8)	χ^2=2.41, p=0.14
	65-75		n=73	n=174	
		QUS T<-2.5, n (%)	9 (12.3)	28 (16.1)	χ^2=0.57, p=0.56
	75<		n=63	n=126	
		QUS T<-2.5, n (%)	24 (38.1)	48 (38.1)	χ^2=0.00, p=1.00
Women	40-64		n=114	n=269	
		QUS T<-2.5, n (%)	30 (26.3)	59 (21.9)	χ^2=0.86, p=0.36
	65-75		n=129	n=306	
		QUS T<-2.5, n (%)	66 (51.2)	142 (46.4)	χ^2=0.82, p=0.40
	75<		n=95	n=235	
		QUS T<-2.5, n (%)	69 (72.6)	173 (73.6)	χ^2=0.34, p=0.89

QUS: quantitative ultrasound. *p < 0.05

Table S4. Multivariate analysis of genotype for QUS T<-2.5 in participants separated into age groups for both genders.

Gender	Age	Variable	Unit	Univariate	multivariate
				OR (95%CI)	OR (95%CI)
Men	40-64	Age	1 increase		1.02 (0.96-1.08)
		BMI	1 increase		0.89 (0.78-1.01)
		FTO(CC&CT)	/TT	0.48 (0.19-1.23)	0.54 (0.21-1.40)
	65-75	Age	1		1.18 (1.04-1.33)
		BMI	1		0.95 (0.84-1.08)
		FTO(CC&CT)	/TT	0.73 (0.33-1.64)	0.83 (0.37-1.90)
	75<	Age	1		1.16 (1.07-1.27)
		BMI	1		0.93 (0.84-1.03)
		FTO(CC&CT)	/TT	1.00 (0.54-1.86)	1.04 (0.54-2.00)
Women	40-64	Age	1		1.13 (1.07-1.19)
		BMI	1		0.88 (0.81-0.96)
		FTO(CC&CT)	/TT	1.27 (0.77-2.11)	1.25 (0.73-2.13)
	65-75	Age	1		1.03 (0.97-1.10)
		BMI	1		0.92 (0.87-0.97)
		FTO(CC&CT)	/TT	1.21 (0.80-1.83)	1.29 (0.85-1.97)
	75<	Age	1		1.07 (1.00-1.15)
		BMI	1		0.94 (0.87-1.02)
		FTO(CC&CT)	/TT	0.95 (0.56-1.63)	0.92 (0.53-1.58)

BMI: body mass index, OR: odds ratio, CI: confidence interval. Logistic regression analysis *P < 0.05

Discussion

We analyzed the associations of the SNP rs1421085 with obesity and bone health among 1,828 adults from Goto city in western Japan. Carriers of FTO rs1421085 CC and CT variant genotypes at all age ranges had higher BMIs, consistent with previous reports. Participants with CC and CC/CT genotypes were associated with a reduced risk of having low bone mass. Interestingly, this association was only observed in middle-aged and older men.

The biological mechanism underlying the association between FTO and bone mass is unknown. In humans, expression of FTO has been found in various tissues such as adipose tissue and beta cells[25], but there is no evidence for its expression in bone. The present finding was based on an association analysis, which does not necessarily mean that FTO is causally linked to bone mass; however, it is likely that these polymorphisms would influence several protective factors that are associated with bone mass liability, such as obesity, muscle, lifestyle, and hormones.

FTO and obesity

FTO has been well described in relation to body composition and obesity phenotypes[26, 27]. Epidemiological surveys have shown that body weight and BMI are positively correlated with bone density, and that weight loss may promote bone loss. However, several studies on Asian and Caucasian populations have shown a negative correlation between obesity and BMD after adjusting for the effect of body weight, suggesting that body fat content may negatively regulate BMD, except for the positive effect of weight bearing[28, 29]. Combined with our results, we suggest that the complex relationship between obesity and osteoporosis may be related to different definitions or measures of obesity.

FTO and muscle

In 2019, Taniguchi, et al. found that loss of skeletal muscle mass was independently associated with osteoporosis after adjusting for covariates[30]. In an animal model, FTO-knockout mice showed a significant reduction in adipose tissue and lean body mass[31], and reduced lean mass was associated with weakened femur bone strength[32]. In 2017, Wang et al.

found that skeletal muscle development was impaired in mice with FTO deficiency, and they demonstrated that FTO might act upstream of the mTOR-PGC-1 α pathway and regulate myocyte differentiation[33]. The CC genotype of the rs1421085 variant was reported to be associated with decreased hand grip strength[34]. These studies laid a foundation for further studies on the molecular mechanism of FTO in myoblast differentiation.

FTO and lifestyle

Several studies have suggested that FTO plays a role in controlling feeding behavior and energy expenditure. FTO variants leading to obesity seem to be related to energy intake[35], increased dietary macronutrient intake[36], and more frequent episodes of loss-of-control eating[37].

In addition to nutrition, sleep habits and quality also have a significant impact on bone health. Short sleep is associated with a low bone mineral density and osteoporosis[38]. Several epidemiological studies support this inference; people whose sleep and circadian rhythms are disrupted because night shift work are often associated with lower bone density and a higher risk of fractures[39]. Sleep duration could enhance the effect of rs1421085 on BMI in United Kingdom[40]. Hence, we speculate that FTO is involved in sleep and may affect bone health through this pathway.

FTO and other factors

Studies have shown that there is a relationship between bone mass and fat metabolism, and that a variety of bioactive molecules secreted by fat, such as estrogen, resistin, leptin, adiponectin, and IL-6, all participate in the process of bone metabolism. Among them, insulin-like growth factor-1 (IGF-I) has been reported to promote bone formation[41]. FTO risk alleles were associated with lower IGF-I levels independent of age and sex[42]. Leptin has been reported to have a direct anabolic effect on osteoblasts and chondrocytes[43]. In a cross-sectional study of 985 older adults, Benedict et al. found that the FTO risk allele was associated with lower serum levels of the satiety-enhancing adipokine leptin and with higher plasma levels of the hunger hormone ghrelin. Importantly, the observed association was independent of obesity[44].

In 2009, Cizza et al. concluded that depression causes bone loss and osteoporotic fractures primarily through specific immune and endocrine mechanisms[45]. Rivera et al. analyzed the interaction between depression, FTO, and BMI in a meta-study of 13,701 individuals. They suggested that the association between high BMI and major depression may be mediated by FTO[46]. In 2019, Sun et al. published an animal study reporting that FTO deficiency reduced behaviors such as anxiety and depression by altering the gut microbiome in mice[47]. It might be possible to speculate that depression, along with other psychological factors, may also play a role in how FTO affects bone health.

No association between FTO genotype and osteoporosis was found in other age groups

We observed an association between FTO polymorphisms and group with QUS T-score less than -2.0 in the 65-75 age men, and the group with QUS T-score less than -1.0 in the 40-64 age men. One possible reason is that osteoporosis is a disease affecting the aging population and rarely occurs in young people, but the effects of genetic factors can accumulate in the bone tissue. The age-dependent decline in FTO transcription expression in muscle was reported[48], opposing this finding, and another study showed that FTO expression in subcutaneous and visceral fat increased with age[25]. These might modify our results.

No association between FTO genotype and osteoporosis was found in women

We observed an association between FTO polymorphism and osteoporosis in men, but not in women, although there was a relatively large number of participants. There are several possible reasons for this. First is the comprehensive influence of multiple endocrine factors. The hormone levels in postmenopausal women change more compared to premenopausal women, which is a confounding factor that could not be ignored in this study. Estrogen withdrawal during menopause also increases the production of inflammatory cytokines, which affect osteoblasts[49, 50].

In addition to the impact of dramatic changes in sex hormones on bone, population studies have shown that reference changes in thyroid status in postmenopausal women are also associated with changes in bone mineral density and fracture risk[51]. A second possibility could be the different types of obesity in men and women. Obesity protects against bone loss, but more recently, it has been recognized that the relationship between obesity and osteoporosis depends on the definition of obesity[52]. Postmenopausal women had five times the chance of having central adiposity than premenopausal women[53]. These may be the reasons why the CC and CT/CC variants of rs1421085 were not found to be associated with a reduced risk of low bone mass in elderly women.

Strengths

There are studies on the association between FTO and obesity, diabetes, tumors, among others. However, few studies have focused on the effect of the FTO genotype on bone

health. Our results might be a clue for the promotion of precision medicine in bone health.

Limitations

Our study was a cross-sectional design of genetic epidemiology, which cannot reflect the molecular pathogenesis of the causal variant. We could not assess whether rs1421085 may be a causal variation or not. Second, there were few CC variants in the population, which interfered with assessing the associations of genotype with phenomena in a gene dosage-dependent manner. Third, it is necessary to apply our results to a different ethnic population because genomic variations are greater when compared between ethnicities. Fourth, we did not have results of bone mineral density obtained from DXA, which is a gold standard for diagnosis of osteoporosis. We could not assess the any association between QUS parameters and diagnosis of osteoporosis or osteopenia. Lastly, we could not eliminate the influence of potential confounding factors such as smoking, nutrition, other diseases, and the medications taken by the participants. This might result in an underestimation of the association between genotype and bone health.

Conclusion

We showed that the obesity-related FTO genotype had an independent protective association against low bone mass among community-dwelling men aged 65-75 years, after adjusting for BMI. We conclude that the polymorphism may play a role in bone health with higher BMI and other beneficial functions.

References

1. Seeman E, Delmas PD. Bone quality--the material and structural basis of bone strength and fragility. N Engl J Med. May 25 2006;354(21):2250-61.
2. Hagino H, Jackson M, Gitlin M, Wessler Z. Estimating the future clinical and economic benefits of improving osteoporosis diagnosis and treatment among women in Japan: a simulation projection model from 2020 to 2040. Arch Osteoporos. 2021 Oct 12;16(1):156.
3. Genant HK, Cooper C, Poor G, Reid I, Ehrlich G, Kanis J, et al. Interim report and recommendations of the World Health Organization Task-Force for Osteoporosis. Osteoporos Int. 1999;10(4):259-64.
4. Soen S, Fukunaga M, Sugimoto T, Sone T, Fujiwara S, Endo N, Gorai I, Shiraki M, Hagino H, Hosoi T, Ohta H, Yoneda T, Tomomitsu T; Japanese Society for Bone and Mineral Research and Japan Osteoporosis Society Joint Review Committee for the Revision of the Diagnostic Criteria for Primary Osteoporosis. Diagnostic criteria for primary osteoporosis: year 2012 revision. J Bone Miner Metab. 2013;31(3):247-57.
5. Bauer DC, Ewing SK, Cauley JA, Ensrud KE, Cummings SR, Orwoll ES; Osteoporotic Fractures in Men (MrOS) Research Group. Quantitative ultrasound predicts hip and non-spine fracture in men: the MrOS study. Osteoporos Int. 2007 Jun;18(6):771-7.
6. Krieg MA, Cornuz J, Ruffieux C, Van Melle G, Büche D, Dambacher MA, Hans D, Hartl F, Häuselmann HJ, Kraenzlin M, Lippuner K, Neff M, Pancaldi P, Rizzoli R, Tanzi F, Theiler R, Tyndall A, Wimpfheimer C, Burckhardt P. Prediction of hip fracture risk by quantitative ultrasound in more than 7000 Swiss women > or =70 years of age: comparison of three technologically different bone ultrasound devices in the SEMOF study. J Bone Miner Res. 2006 Sep;21(9):1457-63.
7. Hans D, Dargent-Molina P, Schott AM, Sebert JL, Cormier C, Kotzki PO, Delmas PD, Pouilles JM, Breart G, Meunier PJ. Ultrasonographic heel measurements to predict hip fracture in elderly women: the EPIDOS prospective study. Lancet. 1996 Aug 24;348(9026):511-4.
8. Fujiwara S, Sone T, Yamazaki K, Yoshimura N, Nakatsuka K, Masunari N, Fujita S, Kushida K, Fukunaga M. Heel bone ultrasound predicts non-spine fracture in Japanese men and women. Osteoporos Int. 2005 Dec;16(12):2107-12.
9. Khaw KT, Reeve J, Luben R, Bingham S, Welch A, Wareham N, Oakes S, Day N. Prediction of total and hip fracture risk in men and women by quantitative ultrasound of the calcaneus: EPIC-Norfolk prospective population study. Lancet. 2004 Jan 17;363(9404):197-202.
10. Moayyeri A, Adams JE, Adler RA, Krieg MA, Hans D, Compston J, Lewiecki EM. Quantitative ultrasound of the heel and fracture risk assessment: an updated meta-analysis. Osteoporos Int. 2012 Jan;23(1): 143-53.
11. Hadji P, Hars O, Wüster C, Bock K, Alberts US, Bohnet HG, Emons G, Schulz KD. Stiffness index identifies patients with osteoporotic fractures better than ultrasound velocity or attenuation alone. Maturitas. 1999 Mar 15;31(3):221-6.
12. Gonnelli S, Cepollaro C, Gennari L, Montagnani A, Caffarelli C, Merlotti D, Rossi S, Cadirni A, Nuti R. Quantitative ultrasound and dual-energy X-ray absorptiometry in the prediction of fragility fracture in men. Osteoporos Int. 2005 Aug;16(8):963-8.
13. Clò A, Gibellini D, Damiano D, Vescini F, Ponti C, Morini S, Miserocchi A, Musumeci G, Calza L, Colangeli V, Viale P, Re MC, Borderi M. Calcaneal quantitative ultrasound (QUS) and dual X-ray absorptiometry (DXA) bone analysis in adult HIV-positive patients. New Microbiol. 2015 Jul;38(3):345-56.
14. Liu YZ, Liu YJ, Recker RR, Deng HW. Molecular studies of identification of genes for osteoporosis: the 2002 update. J Endocrinol. May 2003; 177(2):147-96.
15. Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM, et al. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. Science. May 11 2007;316(5826):889-94.
16. Yamaji T, Iwasaki M, Sawada N, Shimazu T, Inoue M, Tsugane S. Fat mass and obesity-associated gene polymorphisms, pre-diagnostic plasma adipokine levels and the risk of colorectal cancer: The Japan Public Health Center-based Prospective Study. PLoS One. 2020;15(2):e0229005.
17. Kang Y, Liu F, Liu Y. Is FTO gene variant related to cancer risk independently of adiposity? An updated meta-analysis of 129,467 cases and 290,633 controls. Oncotarget. Aug 1 2017;8(31):50987-96.
18. Lurie G, Gaudet MM, Spurdle AB, Carney ME, Wilkens LR, Yang HP, et al. The obesity-associated polymorphisms FTO rs9939609 and MC4R rs17782313 and endometrial cancer risk in non-Hispanic white women. PLoS One. Feb 8 2011;6(2):e16756.
19. Kaklamani V, Yi N, Sadim M, Siziopikou K, Zhang K, Xu Y, et al. The role of the fat mass and obesity associated gene (FTO) in breast cancer risk. BMC Med Genet. Apr 13 2011;12:52.
20. Rivera M, Locke AE, Corre T, Czamara D, Wolf C, Ching-Lopez A, et al. Interaction between the FTO gene, body mass index and depression: meta-analysis of 13701 individuals. Br J Psychiatry. Aug 2017;211(2):70-6.
21. Gao X, Shin YH, Li M, Wang F, Tong Q, Zhang P. The fat mass and obesity associated gene FTO functions in the brain to regulate postnatal growth in mice. PLoS One. Nov 16 2010;5(11):e14005.
22. Claussnitzer M, Dankel SN, Kim KH, Quon G, Meuleman W, Haugen C, et al. FTO Obesity Variant Circuitry and Adipocyte Browning in Humans. N Engl J Med. Sep 3 2015;373(10):895-907.
23. Tomita Y, Arima K, Mizukami S, Tsujimoto R, Kawashiri SY, Nishimura

T, Okabe T, Tanaka N, Honda Y, Nakahara K, Yamamoto N, Ohmachi I, Goto H, Hasegawa M, Sou Y, Horiguchi I, Kanagae M, Abe Y, Nonaka F, Tamai M, Yamanashi H, Nagata Y, Kawakami A, Maeda T, Aoyagi K. Association between self-reported walking speed and calcaneal stiffness index in postmenopausal Japanese women. BMC Geriatr. 2020 ;20(1):466.
24. Cepollaro C, Gonnelli S, Montagnani A, Caffarelli C, Cadirni A, Martini S, Nuti R. In vivo performance evaluation of the Achilles Insight QUS device. J Clin Densitom. 8(3) (2005) 341-6.
25. Klöting N, Schleinitz D, Ruschke K, Berndt J, Fasshauer M, Tönjes A, et al. Inverse relationship between obesity and FTO gene expression in visceral adipose tissue in humans. Diabetologia. Apr 2008;51(4):641-7.
26. Wheeler E, Huang N, Bochukova EG, Keogh JM, Lindsay S, Garg S, et al. Genome-wide SNP and CNV analysis identifies common and low-frequency variants associated with severe early-onset obesity. Nat Genet. May 2013;45(5):513-7.
27. Do R, Bailey SD, Desbiens K, Belisle A, Montpetit A, Bouchard C, et al. Genetic variants of FTO influence adiposity, insulin sensitivity, leptin levels, and resting metabolic rate in the Quebec Family Study. Diabetes. Apr 2008;57(4):1147-50.
28. Zhao LJ, Liu YJ, Liu PY, Hamilton J, Recker RR, Deng HW. Relationship of obesity with osteoporosis. J Clin Endocrinol Metab. May 2007;92(5): 1640-6.
29. Hsu YH, Venners SA, Terwedow HA, Feng Y, Niu T, Li Z, et al. Relation of body composition, fat mass, and serum lipids to osteoporotic fractures and bone mineral density in Chinese men and women. Am J Clin Nutr. Jan 2006;83(1):146-54.
30. Taniguchi Y, Makizako H, Kiyama R, Tomioka K, Nakai Y, Kubozono T, et al. The Association between Osteoporosis and Grip Strength and Skeletal Muscle Mass in Community-Dwelling Older Women. Int J Environ Res Public Health. Apr 6 2019;16(7).
31. Fischer J, Koch L, Emmerling C, Vierkotten J, Peters T, Brüning JC, et al. Inactivation of the Fto gene protects from obesity. Nature. Apr 16 2009;458(7240):894-8.26.
32. Travison TG, Araujo AB, Esche GR, Beck TJ, McKinlay JB. Lean mass and not fat mass is associated with male proximal femur strength. J Bone Miner Res. Feb 2008;23(2):189-98.
33. Wang X, Huang N, Yang M, Wei D, Tai H, Han X, et al. FTO is required for myogenesis by positively regulating mTOR-PGC-1 α pathway-mediated mitochondria biogenesis. Cell Death Dis. Mar 23 2017;8(3):e2702.
34. Tikkanen E, Gustafsson S, Amar D, Shcherbina A, Waggott D, Ashley EA, et al. Biological Insights Into Muscular Strength: Genetic Findings in the UK Biobank. Sci Rep. Apr 24 2018;8(1):6451.
35. Cecil JE, Tavendale R, Watt P, Hetherington MM, Palmer CN. An obesity-associated FTO gene variant and increased energy intake in children. N Engl J Med. Dec 11 2008;359(24):2558-66.
36. Tanaka T, Ngwa JS, van Rooij FJ, Zillikens MC, Wojczynski MK, Frazier-Wood AC, et al. Genome-wide meta-analysis of observational studies shows common genetic variants associated with macronutrient intake. Am J Clin Nutr. Jun 2013;97(6):1395-402.
37. Tanofsky-Kraff M, Han JC, Anandalingam K, Shomaker LB, Columbo KM, Wolkoff LE, et al. The FTO gene rs9939609 obesity-risk allele and loss of control over eating. Am J Clin Nutr. Dec 2009;90(6):1483-8.
38. Ochs-Balcom HM, Hovey KM, Andrews C, Cauley JA, Hale L, Li W, et al. Short Sleep Is Associated With Low Bone Mineral Density and Osteoporosis in the Women's Health Initiative. J Bone Miner Res. Feb 2020;35(2):261-8.
39. Quevedo I, Zuniga AM. Low bone mineral density in rotating-shift workers. J Clin Densitom. Oct-Dec 2010;13(4):467-9.
40. Young AI, Wauthier F, Donnelly P. Multiple novel gene-by-environment interactions modify the effect of FTO variants on body mass index. Nat Commun. 2016 Sep 6;7:12724.
41. Fisher MC, Meyer C, Garber G, Dealy CN. Role of IGFBP2, IGF-I and IGF-II in regulating long bone growth. Bone. Dec 2005;37(6):741-50.
42. Rosskopf D, Schwahn C, Neumann F, Bornhorst A, Rimmbach C, Mischke M, et al. The growth hormone--IGF-I axis as a mediator for the association between FTO variants and body mass index: results of the Study of Health in Pomerania. Int J Obes (Lond). Mar 2011;35(3):364-72.
43. Reid IR, Baldock PA, Cornish J. Effects of Leptin on the Skeleton. Endocr Rev. Dec 1 2018;39(6):938-59.
44. Benedict C, Axelsson T, Söderberg S, Larsson A, Ingelsson E, Lind L, et al. Fat mass and obesity-associated gene (FTO) is linked to higher plasma levels of the hunger hormone ghrelin and lower serum levels of the satiety hormone leptin in older adults. Diabetes. Nov 2014;63(11): 3955-9.
45. Cizza G, Primma S, Csako G. Depression as a risk factor for osteoporosis. Trends Endocrinol Metab. Oct 2009;20(8):367-73.
46. Rivera M, Locke AE, Corre T, Czamara D, Wolf C, Ching-Lopez A, et al. Interaction between the FTO gene, body mass index and depression: meta-analysis of 13701 individuals. Br J Psychiatry. 2017 Aug;211(2):70-76.
47. Sun L, Ma L, Zhang H, Cao Y, Wang C, Hou N, et al. Fto Deficiency Reduces Anxiety- and Depression-Like Behaviors in Mice via Alterations in Gut Microbiota. Theranostics. 2019;9(3):721-33.
48. Grunnet LG, Nilsson E, Ling C, Hansen T, Pedersen O, Groop L, et al. Regulation and function of FTO mRNA expression in human skeletal muscle and subcutaneous adipose tissue. Diabetes. Oct 2009;58(10):2402-8.
49. Cenci S, Toraldo G, Weitzmann MN, Roggia C, Gao Y, Qian WP, et al. Estrogen deficiency induces bone loss by increasing T cell proliferation and lifespan through IFN-gamma-induced class II transactivator. Proc Natl Acad Sci U S A. Sep 2 2003;100(18):10405-10.
50. Hannan MT, Felson DT, Dawson-Hughes B, Tucker KL, Cupples LA, Wilson PW, et al. Risk factors for longitudinal bone loss in elderly men and women: the Framingham Osteoporosis Study. J Bone Miner Res. Apr 2000;15(4):710-20.
51. Williams GR, Bassett JHD. Thyroid diseases and bone health. J Endocrinol Invest. Jan 2018;41(1):99-109.
52. Migliaccio S, Greco EA, Fornari R, Donini LM, Lenzi A. Is obesity in women protective against osteoporosis? Diabetes Metab Syndr Obes. 2011;4:273-82 PubMed .
53. Donato GB, Fuchs SC, Oppermann K, Bastos C, Spritzer PM. Association between menopause status and central adiposity measured at different cutoffs of waist circumference and waist-to-hip ratio. Menopause. 2006 Mar-Apr;13(2):280-5.